A Microbiological Process Report

Aerobic Treatment of Dairy Wastes

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Disposal of dairy wastes is of importance to all branches of the dairy industry, from the simple shipping and bottling of milk through the manufacture of milk products, including cheese, butter, ice cream, and processed milk. Drainage and washings are the main sources of wastes, with an occasional addition of surplus buttermilk, whey or spoiled materials. About 1 per cent of fluid milk entering a plant is lost even under good waste-preventing practices. Wash and cooling waters dilute this milk so that about 0.1 to 0.2 per cent milk solids is in the waste. This industrial waste with a 5-day biochemical oxygen demand (BOD) of 800 to 1500 parts per million (ppm) is a much stronger polluting agent than the same volume of domestic sewage with a BOD of about 200 ppm.

Increasing demands for clean streams, failure of existing aeration systems to prevent odor and acid formation in dairy wastes, and the need for simplified small disposal systems induced the Dairy Industry Committee to present the problem to the Department of Agriculture.

The low concentrations of solids in dairy waste does not warrant costly installation for anaerobic disposal. The relatively great oxygen demand, as well as the ready biochemical availability of the major constituents, lactose and protein, suggested studying an aeration treatment. Aerated or activated sludge processes for waste purification use microorganisms for the conversion of soluble oxygen-demanding organic materials to innocuous substances. Aerobic activities of the sludge microorganisms were investigated in these studies, in which a 0.1 per cent solution (1000 ppm) of dried skim milk was used as synthetic waste (table 1) having the same polluting strength as dairy waste.

Rapid changes resulting from microbial activity in an aeration process could not be followed satisfactorily by the cumbersome and slow BOD test. Chemical oxygen demand (COD) determined by bichromate oxidation proved valuable in these studies. Details of this procedure, which gives results in a few minutes equivalent to the 20-day BOD of milk wastes, were published (Porges *et al.*, 1950). In addition, factors were determined for estimating the pollution load or oxygen demands of substances. For lactose, casein, and sludge microbial cells, these were 1.12, 1.42, and 1.25, respectively. Thus, 100 mg dried bacterial cells required an average of 125 mg oxygen for complete combustion, or, conversely, multiplying the COD of cells by 0.8 gave the weight of cells in the suspension.

Pure culture studies. Single strains of microorganisms were found undesirable for oxidizing dairy waste (Porges *et al.*, 1950). Simulated waste was inoculated and aerated for 96 hr. *Bacillus polymyxa* caused the protein to disappear. Lactose was also used, with a reduction of 37 per cent in the total COD. These components were hydrolyzed, as shown by an accumulation of soluble nitrogenous and other substances. An unidentified organism also produced nonprotein nitrogenous substances and left the lactose undisturbed, with no decrease in COD. *Saccharomyces fragilis*, a feed yeast grown on whey (Enebo *et al.*, 1941; Porges *et al.*, 1951) converted the protein and lactose to cell material, causing a reduction of 26 per cent in COD. This reduction reached 75 per cent when the solids were removed.

TABLE 1. *Comparison between whole milk and dried skim milk*

	WHOLE MILK	DRIED SKIM MILK	MILK SOLUTION*	SKIM MILK SOLUTION*
	%	%	*ppm*	*ppm*
Lactose	5.1	50.5	567	529
Protein	3.2	36.9	355	386
Fat	3.9	0.9	—	—
Ash	0.7	8.1	78	85
Total solids	12.9	96.4	1000	1000
Organic solids	12.2	88.3	922	915

* Calculated as 1000 ppm total solids, without fat and water.

Studies are continuing on the identification of bacteria found in an aerated dairy waste sludge and on determination of their biochemical activity on milk components. However, the impracticability of using pure culture conditions on a valueless waste was obvious, and experiments were continued with the mixed culture that occurs naturally in aerated sludge.

Continuous flow process. Simulated waste was fed into an aerobic fermenter (Humfeld, 1947) at a constant

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rate while the effluent drained continuously from an overflow level at the 20-liter mark. Temperature was maintained at 30 C and air passed through the tank at 20 L per min. The rates of addition were 1.0, 1.5 and 2 L per hr, giving an apparent holding time of 20, 13.3 and 9.5 hrs, respectively (Hoover and Porges, 1949). Reductions of 40 to 50 per cent in total COD were attained (figure 1), and after centrifugal separation of the solids the COD of the effluent was only 2 to 11 per cent of the influent. Throughout this experiment, lactose was absent from the effluent; the protein content was equivalent to the total nitrogen content of the influent.

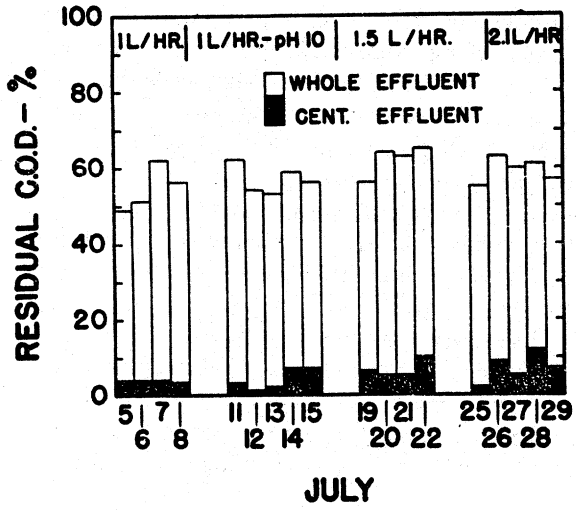

FIG. 1. Removal of COD by sludge under continuous feed and aeration. (*Reprinted from the Proceedings of the 5th Industrial Waste Conference.*)

TABLE 2. *Composition of microbial solids recovered in a centrifuge (moisture-free)*

RECOVERY DATE	PROTEIN	CARBOHYDRATE	ASH
July 8	66.3	13.6	7.1
July 15	68.4	14.8	6.5
July 25	69.7	8.6	10.6
July 29	65.3	15.5	7.2
Average	67.4	10.5	7.9

Micro-organisms oxidized or converted the soluble organic material to products removable by aeration or by gravity, thus leaving an effluent low in COD and much lower in the 5-day BOD.

Obviously the proteins and carbohydrate materials stabilized in the cell were the COD substances. The dried centrifuged solids were found high in proteins. The proteins, carbohydrates and ash approximated 86 per cent of the cell weight (table 2).

A balance of the organic solids was obtained from these results and the original data on the simulated waste. Air-dried skim milk containing 53 parts lactose and 35 parts protein (calculated from total nitrogenous matter) made 88 parts of organic material available for microbial attack. A solids balance sheet for aerobic assimilation was prepared (table 3) from the protein and carbohydrate contents of the cells and those of the clear effluent. Thus, when 0.1 per cent solution of skim milk was fed to a vigorously aerated microbial sludge, all the nitrogen was utilized for cell synthesis, and about 83 per cent of the lactose was completely oxidized, leaving only about one-half as much of the solids as were originally present.

Fill-and-draw process. Continuous flow systems are impracticable for many small dairies that obtain the

TABLE 3. *Solids balance sheet for aerobic assimilation of milk solids by sludge organisms under continuous feed and aeration*

	PROTEIN	CARBOHYDRATE	TOTAL
Influent solubles	35*	53	88
Effluent solids	34	7	41
Effluent solubles	1	2	3
Material oxidized	0	44	44

* Per cent of air dried skim milk.

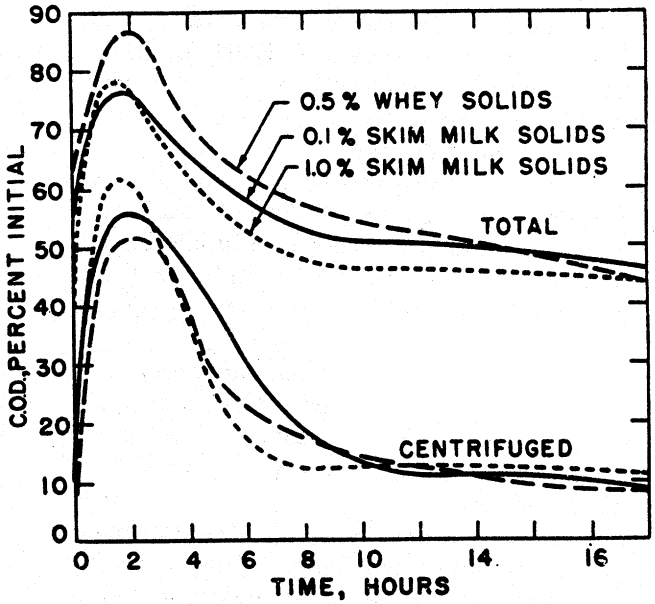

FIG. 2. Increase and decrease of COD in fill and draw operations. (*Reprinted from the Proceedings of the 6th Industrial Waste Conference.*)

greater proportion of the waste during the morning hours. Therefore, experiments were made on a fill-and-draw system in which four-fifths of the aerated material was withdrawn while being aerated and replaced in 4 hr by fresh waste (Hoover *et al.*, 1951b). Thus 9.6 L of simulated waste was added to 2.4 L of aerating inoculum. The COD value was determined hourly. The mechanical action of the stirring blades in this type aerator produced a light, finely divided dispersion of cells that did not have the settling characteristics of activated sludge.

Figure 2 presents the average data of a series of ex-

periments made on different simulated wastes containing 0.1 and 1.0 per cent skim milk solids and 0.5 per cent whey solids. The COD of the tank contents increased rapidly, but started to drop within the 4 hr that the waste was added. The COD values of the soluble substances rose simultaneously with the total COD, showing that the waste was not used as rapidly as it was being added during the early feeding period. In about 6 to 8 hours, the rapid oxidation was completed, but a slower oxidation of the bacterial cells continued, causing a slight decrease in the COD.

The values in figure 2 were plotted as the percentage of the initial COD of the added simulated milk waste. The total COD was reduced about 55 to 60 per cent;

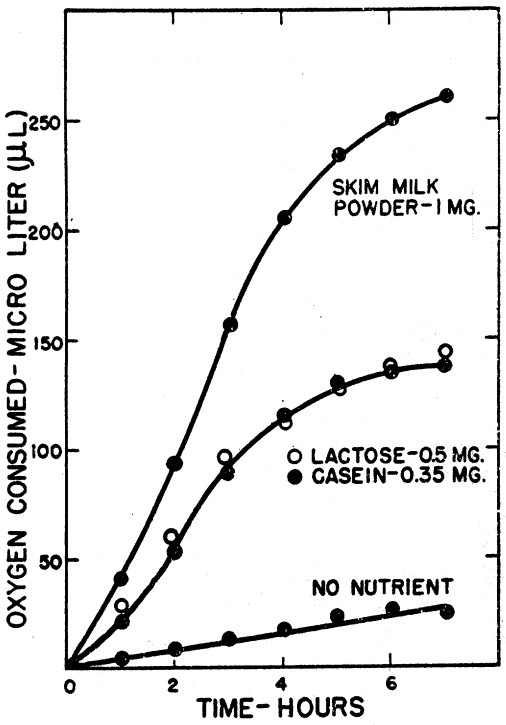

FIG. 3. Oxidation of skim milk, casein and lactose by 1.0 ml sludge mixture (measured manometrically). (*Reprinted from Sewage and Industrial Waste.*)

the cell-free supernatant had only about 10 per cent of the original COD. Further analyses showed a reduction of 75 to 80 per cent in the 5-day BOD. The BOD and COD values of the effluent after the removal of the cells were low and indicated 89 to 95 per cent removal of the original oxygen demanding substances. These tests showed that a fill-and-draw method may be applied where wastes are produced intermittently. The microorganisms rapidly oxidized or assimilated the soluble organic matter, leaving a relatively stable mixture amenable to further simple treatment such as separation in a municipal sewage plant.

Oxidation of dairy waste. Inasmuch as carbon dioxide, water and microbial substance were apparently the main products, a detailed study of the oxidative changes were made manometrically (Hoover *et al.*, 1951a). Manometric methods had been proposed for the determination of BOD (Gellman and Heukelekian, 1951), since carbon dioxide was the only gas produced in this oxidation (Wooldridge and Standfast, 1936). The effects of a number of variables on the oxygen requirements of activated sludge have been measured (Dawson and Jenkins, 1949). The present studies with skim milk, lactose and casein were made in a standard Warburg respirometer. Procedures described in standard published works were used, although the use of 125-ml flasks had been proposed (Caldwell and Langelier, 1948).

A mixed sludge, developed in the aerobic fermentor by feeding continuously a 1000-ppm skim milk solution, was placed directly in Warburg vessels in 1 ml quantities. The desired amount of substrate dissolved in 1 ml phosphate buffer (pH 6.9) was added from the side arm. Nitrogen was supplied as ammonium sulfate to compensate for its absence in lactose. Oxidation of skim milk and its principal components was measured (figure 3). The similar rate and extent of oxidation of

TABLE 4. *Oxygen utilization by organisms in the presence of skim milk, lactose and casein*

	SUBSTRATE	THEORETICAL VALUE	OBSERVED VALUE*	PERCENTAGE USED
	mg	*µl*	*µl*	%
Skim milk	1.00	780	250	32
Lactose	0.50	371	136	37
Casein	0.35	335	133	40

* (determined manometrically over 6 hours)

500 ppm lactose and 350 ppm casein occurred repeatedly. The skim milk was oxidized at a rate almost equal to that of the sum of its components. Endogenous respiration of the culture without nutrients was low and practically constant, showing oxidation of cell constituents for maintenance energy. Addition of nutrients induced high rates of oxidation for a few hr, which dropped in about 6 hr to that of the control. The actual rates of oxidation were calculated to absolute values in terms of microliters oxygen used per mg dry cells per hr. This value or Q_{O_2} was 7 to 18 for unfed cells, 62 to 116 for casein, 60 to 114 for lactose and 106 to 183 for skim milk.

Only 32 to 40 per cent of the theoretical amount of oxygen necessary for complete oxidation was required (table 4), indicating the occurrence of either partial oxidation or assimilation or both. The theoretical values for complete oxidation were calculated from the mole composition of the substrate; thus 0.5 mg lactose hydrate would require 530 µg C_2 or 371 µl. Casein would require 1.48 mg O_2 or 1035 µl per mg if the nitrogen were converted to NH_3, or 335 µl O_2 for 0.35 mg air-dry casein (8 per cent moisture). The percentage composition of

the casein was: C, 53; H, 7.0; N, 15.7; and O, 27.7. Since the skim milk contained 50 per cent lactose anhydride and 36.3 per cent protein, the remainder being primarily ash and water, calculation gave a value of 1000 μg or 780 μl O_2 per mg air-dried product.

Respiratory quotient. Measurement of the absorbed CO_2 in the respirometer confirmed the supposition that substrate was assimilated or oxidized. The respiratory quotient (RQ) of the systems showed that the volume of carbon dioxide evolved was almost equal to the volume of oxygen used. Thus, the RQ (CO_2/O_2) for the assimilation reaction was 1.00 for casein, 1.03 for skim milk and 1.04 for lactose. These values happen to be almost equal to the theoretical values for complete oxidation of 0.96, 1.00 and 1.00, respectively. In these cases, one mole of CO_2 was produced for each mole of O_2 used.

The RQ of microbial reactions is of value not only for the direct measurement of the conversion of oxygen to carbon dioxide but also in establishing the equations of the reactions when the substrate and nonvolatile reaction products are known. For example, the complete oxidation of ethanol to carbon dioxide and water has an RQ of 0.67, whereas the oxidation of ethanol to acetic acid requires oxygen and produces no carbon dioxide, giving an RQ of zero. Apparently in these experiments having an RQ of 1.0, the unassimilated substrate was completely oxidized, and the primary reaction was: Milk solids + O_2 → microbial cells + CO_2 + H_2O.

Oxygen depletion. Polarographic methods may be used for direct determinations of dissolved oxygen. A modification of the method of Hixon and Gaden (1950) was used to determine the relative rate of oxidation by the assimilative reaction and by endogenous respiration and the effect of low concentration of oxygen on these two rates. Rates of oxygen depletion by bacterial sludge fed various concentrations of nutrients were determined by continuous recording polarograms (Hoover and Porges, 1952).

Figure 4 shows curves representing oxygen depletion after cessation of aeration of aerated sludges containing 500 ppm cell solids to which skim milk was added. There was a rapid and relative constant rate of oxidation. Plotting the rate of oxidation as a function of substrate concentration permitted more satisfactory interpretation of the data.

Values in figure 5 were obtained by approximating the time required to reduce the oxygen tension to 0.5 ppm. The most rapid rate of depletion at 1000 ppm (figure 4) was taken as unity. The rate of assimilation was essentially constant above 100 ppm skim milk with the amount of microorganisms used, showing that the nutrient was not a limiting factor above 100 ppm concentration. The time required to oxidize the waste was directly proportional to the amount of skim milk added in this upper range; therefore, since the respirometer studies showed that 1000 ppm were converted in 6 hr, 500 ppm would be used in 3 hr, 250 ppm in 1.5 hr, and other amounts in similar proportions. These calculations assumed a sufficiency of oxygen. In these experiments, the rate of oxygen utilization during assimilation was 6 times the rate of the endogenous control without substrate, as compared with vigorous aeration experiments in which the endogenous rate was one-tenth or less than the assimilation rate.

When the rate of oxidation of 100 ppm milk solids was studied as a function of oxygen concentration, it was found that the rate fell appreciably below 0.5 ppm oxygen concentration. This absolute rate of oxygen consumption is of practical importance. Oxygen was removed from the solution at a rate of 0.35 ppm per

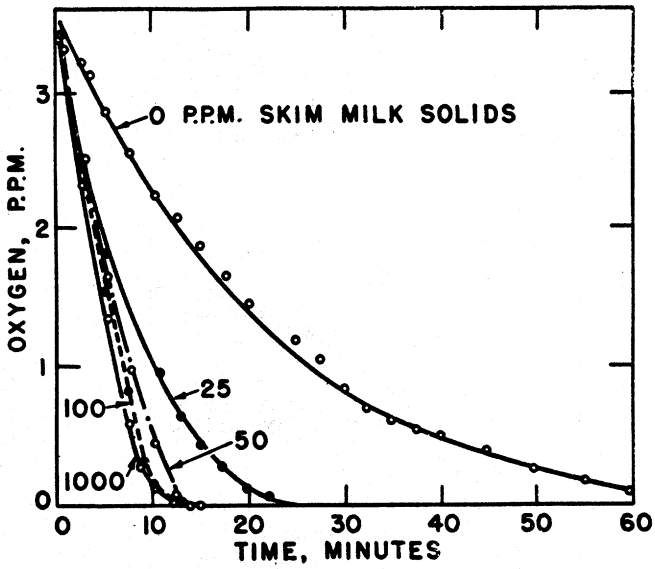

FIG. 4. Oxygen removal by aerobic organisms from solutions of indicated skim milk solids upon cessation of aeration. (*Reprinted from Sewage and Industrial Waste.*)

minute. Supplying oxygen at such a rate is a major problem in the aerobic treatment of dairy wastes.

Oxygen consumption. Direct manometric measurements of oxygen consumption showed a maximum rate of 83 ppm per hour during assimilation, as compared with 22 ppm per hour found by polarographic determination. The vigorous agitation of the culture in the manometric apparatus and the consequent lessened effect of diffusion may be the cause of this difference. If oxygen is supplied rapidly enough, the rate in an aeration tank may equal the rate in these manometer experiments. Another point of interest was the decreased rate of oxygen uptake at the end of 18 hours' aeration. It was then but 7 ppm per hour. The air necessary during the latter stages was much less than the maximum required during the first few hours.

Figure 6 presents data obtained when 400 ml of simulated waste (1000 ppm skim milk solids) were

added in a fill-and-draw operation to 100 ml aerated sludge containing 380 ppm cell solids. The rate of oxidation was determined from the CO_2 evolution and

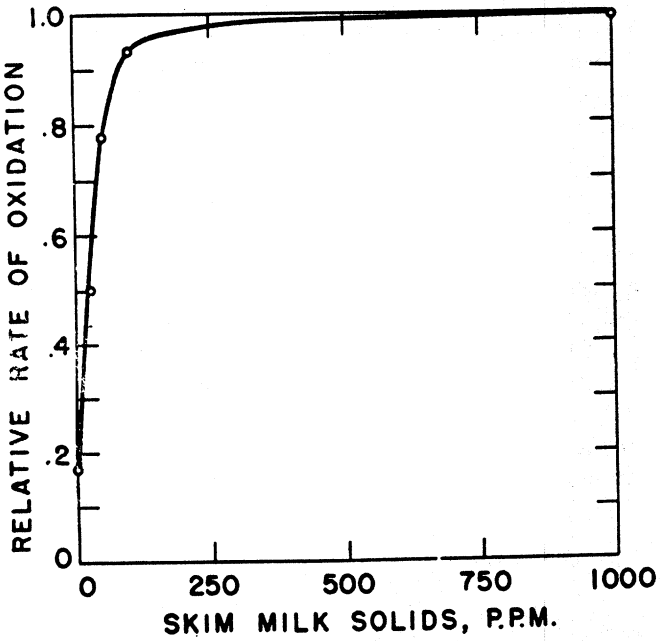

FIG. 5. Rate of oxidation by aerated sludge as a function of milk solids concentration. Values based upon time required to reduce the oxygen concentration to 0.5 ppm. Rate of maximum substrate concentration (1000 ppm) taken as unity. (*Reprinted from Sewage and Industrial Waste.*)

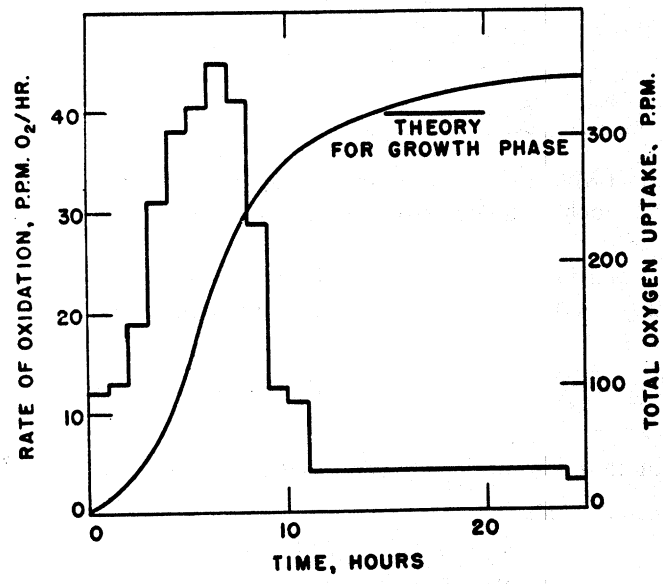

FIG. 6. Oxidation of 800 ppm skim milk solids in a mixture initially containing 76 ppm cell solids. (*Reprinted from Se -age and Industrial Waste.*)

calculated to O_2 uptake per liter of aerating liquor, as discussed in the section on stability of aerated sludge. Addition of 4 volumes of strong waste to 1 volume of the dilute sludge gave a lower rate of oxidation than observed previously, for there were not enough organisms (76 ppm in final mixture) to produce a maximum rate when calculated on a volume basis. If the results were plotted on an absolute dry weight basis or Q_{O_2}, the activity of the cells was high.

The continuous cumulative curve plotted against the right side of figure 6 showed agreement with the theoretical analysis of the reactions. The simulated waste contained 400 × 0.88 (correction for ash and moisture), or 352 mg protein and lactose. According to equations discussed later, this amount of skim milk would use 352:424 = x:192 or x = 159 mg O_2 per 500 ml, or 318 ppm O_2. As observed, when the cumulative oxygen uptake totaled about 300 ppm, the rate of oxidation fell from that characteristic of the rapid phase to that of the slow phase, in excellent agreement with calculated values.

Endogenous respiration for the whole 11- to 23-hour period was at the rate of 4 ppm O_2 per hour. Analysis for organisms showed the presence of 570 ppm organic solids, yet the rate of oxidation was only 3 ppm O_2 per hour. This low rate would cause only a low oxygen drain on the stream. The continuous aeration of dairy waste during a fill-and-draw procedure, with regulated discharge of the whole effluent, may be an effective treatment.

Assimilation for cell growth. The almost complete absence of soluble oxidizable substances led to an investigation of their assimilation for cell growth (Hoover *et al.*, 1951a). The quantity of materials was increased 3-fold; measurements were made in the respirometer for 6 hr, then the contents of the vessels were chilled and centrifuged cold. The COD values of the supernatants were 60 to 96 ppm in 4 vessels, somewhat less than the 118 ppm originally present in the supernatant of the culture, showing removal of the added nutrients. The calculated oxidation was 43 per cent for lactose and casein, and 37 per cent for skim milk, somewhat higher than obtained previously. These results were interpreted as evidence of assimilation of the remaining 57 to 63 per cent of the protein and carbohydrate, an assimilation somewhat higher than the 50 per cent obtained in the continuous flow experiment.

Assimilation by the sludge organisms was comparable with that by microorganisms observed previously. A legume nodule bacterium grown on synthetic media showed an efficiency of about 51 per cent (Hoover and Allison, 1940). Oxidative assimilation of the carbon of carbohydrates was greater than the amount liberated as carbon dioxide in short-time experiments (Clifton, 1946). Two carbon atoms of glucose were oxidized to carbon dioxide in yeast production, and the remaining 4 carbon atoms formed part of the yeast substance (White, 1948). Comparable results were reported for lactose and peptone utilization by activated sludge (Jenkins and Wilkinson, 1940). BOD

removal of 37 per cent was obtained in a dairy waste aeration plant (McKee, 1950). The aerated sludge process of organic waste disposal is based essentially on aerobic microbial assimilation of part of the waste while part is oxidized for energy.

Composition of cells. Detailed consideration of the system required chemical analyses of the cell solids. Protein, carbohydrate and ash were previously determined, but in order to obtain an empirical composition of the microorganisms, direct analyses were made for oxygen, carbon, hydrogen, nitrogen and ash. (As far as is known, these are the first direct determinations for oxygen in micro-organisms.) By dividing the percentage of each element present by its atomic weight, a value for the resultant composition was obtained (Hoover and Porges, 1952).

The empirical formula of these sludge organisms was expressed as $C_5H_7NO_2$ (table 5). This was an obvious over-simplification of the organized system of the microbial cell with its infinite complexity, but the

TABLE 5. *Empirical composition* of mixed microbial cells from aerated sludge*

COMPONENT	PROPORTION	RATIO OF ATOMS	
		Per cent/atomic weight	Per cent/atomic weight†
	%		
C	47.26	3.94	4.9
H	5.69	5.65	7.0
N	11.27	0.81	(1.0)†
O	27.0	1.69	2.1
Ash	8.61	—	—
Total	99.83	—	—

* Empirical formula = $C_5H_7NO_2$.
† N considered as a single atom.

formula did express the average proportions of the major atoms of the organic constituents. Thus, this $C_5H_7NO_2$ unit had a "mole weight" of 113 which, when corrected for the ash content, was 124 atom units. For comparative purposes, yeast had an empirical formula of $C_{13}H_{20}N_2O_7$, calculated from an average analysis.

Cell synthesis from lactose. Oxidation of a portion of this carbohydrate produced energy for the conversion of the remaining portion to cell tissue. Absence of significant amounts of by-products showed that lactose was completely oxidized in this energy-yielding step. Thus

$$C_{12}H_{22}O_{11}\cdot H_2O + 12O_2 \rightarrow 12\ CO_2 + 12H_2O \quad (1)$$

which may be expressed for convenience:

$$(CH_2O) + O_2 \rightarrow CO_2 + H_2O \quad (2)$$
$$RQ = 1/1 = 1.0$$

Conversion of lactose without consumption of oxygen or evolution of carbon dioxide in the presence of ammonia was expressed as follows to yield the empirical cell.

$$5(CH_2O) + NH_3 \rightarrow C_5H_7NO_2 + 3H_2O \quad (3)$$

According to manometric data, only 37 per cent of the theoretical amount of oxygen required for complete oxidation was used during assimilation. Therefore, 5 units of sugar were assimilated and 3 units were oxidized, giving from equations 2 and 3 the following.

$$8CH_2O + 3O_2 + NH_3 \rightarrow C_5H_7NO_2 + 3CO_2 + 6H_2O \quad (4)$$
$$RQ = 3/3 = 1.0$$

The experimental RQ of 1.04 agreed well with the theoretical value. Data on yield by weight were also consistent; 240 atom units, 8 (CH_2O), were used, yielding 124 units of microbial tissue, or 52 per cent by weight.

Cell synthesis from casein. The empirical formula of the casein molecule was $C_8H_{12}N_2O_3$ with a mole weight of 184, as found by direct determination of the 4

TABLE 6. *Empirical composition* of casein*

COMPONENT	PROPORTION	RATIO OF ATOMS	
		%/atomic weight	%/atomic weight†
	%		
C	52.85	4.40	8.2
H	6.48	6.43	11.9
N	15.12	1.08	(2.0)†
O	24.76	1.55	2.9
Total	99.21	—	—

* Empirical formula = $C_8H_{12}N_2O_3$.
† N considered as a single atom.

elements (table 6). The 0.8 per cent sulfur and phosphorus, which equalled only 0.05 atom per mole, was neglected.

The equation for complete oxidation of the casein molecule, if nitrogen was converted to ammonia, may be written

$$C_8H_{12}N_2O_3 + 8O_2 \rightarrow 8CO_2 + 2NH_3 + 3\ H_2O \quad (5)$$
$$RQ = 8/8 = 1.0$$

Assimilation of casein into cell tissue may be equated

$$C_8H_{12}N_2O_3 + 3O_2 \rightarrow C_5H_7NO_2 + NH_3 + 3CO_2 + H_2O \quad (6)$$
$$RQ = 3/3 = 1.0$$

This equation assumed that one-half the nitrogen was assimilated and one-half released as free ammonia. Qualitative evidence in large aerator studies showed the release of ammonia when cells were grown on casein alone. Ammonia was apparent in the gas phase, and the pH of the solution increased, but the amount of ammonia produced was not measured. The oxygen re-

quired in manometric studies for assimilation of casein from buffered solution was three-eighths that of complete oxidation, and the RQ was explainable by these equations.

Cell synthesis from skim milk. The assimilation equations of lactose and casein were compared. The "mole" of cell substance was produced from 240 units of sugar and from 184 units of casein. The proportion of 240:184 was essentially the proportions of lactose to casein found in skim milk. Equations 4 and 6 were added and gave

$$8(CH_2O) + C_8H_{12}N_2O_3 + 6O_2 \rightarrow 2C_5H_7NO_2 + 6CO_2 + 7H_2O \qquad (7)$$
$$RQ = 6/6 = 1.0$$

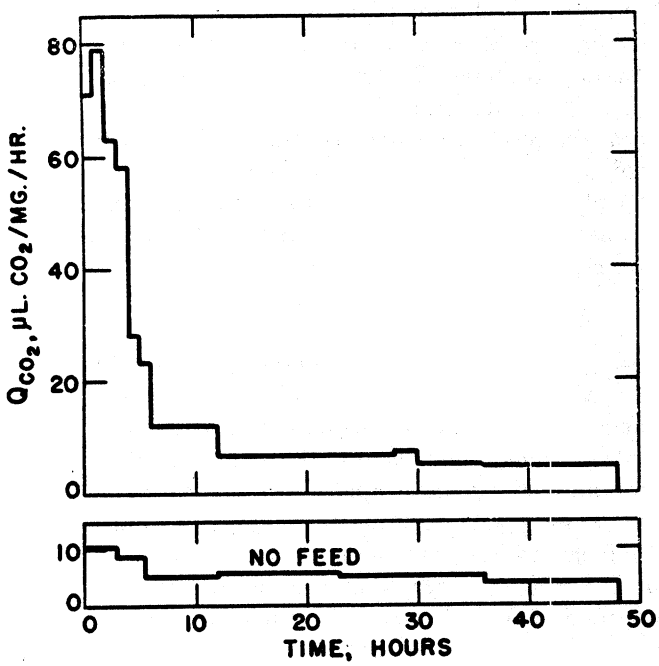

FIG. 7. Rate of CO_2 formation (or oxygen removal) in oxidation of 1000 ppm skim milk. (*Reprinted from Sewage and Industrial Waste.*)

The summation equation showed that only six-sixteenths or 37.5 per cent of the total oxygen needed for complete oxidation was required; the protein supplied the ammonia for carbohydrate assimilation. No change in pH was observed when skim milk was the substrate. According to equation 7, skim milk would yield $2 \times 124/(240 + 184)$ or 58.5 per cent of its weight as microbial tissue. This was greater than the 52 per cent observed in the continuous flow experiments (Hoover and Porges, 1949; Hoover *et al.*, 1951b), where apparently a less complete conversion to cells, carbon dioxide and water was accomplished. If conditions do not permit rapid growth, the yield of cell tissue is lower, for organisms continue to oxidize the sugar and protein according to equations 1 and 5 but at a lower rate, thus increasing the proportion of substrate oxidized to carbon dioxide and water.

Endogenous respiration. Cells produced by the synthetic reactions described above have an endogenous respiration, that is, oxidation of their own tissue for energy, thus

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O \qquad (8)$$
$$RQ = 5/5 = 1.0$$

The digestion of organisms by their own respiration has a definite practical importance. If endogenous respiration proceeds at a great enough rate, microorganisms oxidize their own tissue (autodigestion of sludge) rapidly enough to keep the system in balance. Under such conditions, sludge does not accumulate. If autodigestion is not sufficient, sludge accumulates, making sludge disposal necessary.

Stability of aerated sludge. Rates of endogenous respiration have further importance because of a suggested partial treatment process for small dairy plants permitting discharge to a stream of the aerated mixture (Hoover *et al.*, 1951b). The drain on the oxygen supply of the stream would depend upon the rate of endogenous respiration; hence further data were obtained on rates of oxidation. Carbon dioxide evolution was measured by a simple titrimetric method (Porges *et al.*, 1952) adapted from studies with soil microorganisms (Fred and Waksman, 1928). A comparative study made between the titrimetric and manometric methods showed the microliters carbon dioxide produced per mg cells (Q_{CO_2}) were practically equal to the Q_{O_2}. Also, the rate of oxidation of milk solids was approximately 10 times that of endogenous respiration; determined manometrically the former was 32.1 ml/l/hr and the latter was 3.2 ml/l/hr during the 6-hr period's measurements as compared with 30.3 and 3.9 by titration.

Carbon dioxide evolved from 500-ml aerated solutions containing 410 ppm sludge solids and 1000 ppm skim milk was measured over a period of 48 hr. Samples of the aerated mixture were removed, centrifuged solids were determined by COD, and calculations were made to an absolute basis. Assimilation was high during the first hr (figure 7), being completed by the fifth to sixth hr, when the rate fell to that of the unfed sample. During the remainder of the test, the fed and unfed samples had about the same Q_{CO_2}, the latter dropping from 10 to 4. Q_{O_2}, and Q_{CO_2} values of 8 to 12 for endogenous respiration were obtained in a number of other experiments over the 6-hr period; hence a Q_{O_2} of 10 was considered an average value, although later studies showed slower rates.

An application of the data. The following calculations based on 1 pound of skim milk solids in 1000 pounds of water or 1000 ppm may be of importance in the design and operation of a dairy waste disposal plant.

Assimilation phase:

Part of total O_2 required	37.5	per cent
Quantity of O_2 needed	0.453	lb
Time required	6.	hr
Hourly utilization of O_2	0.075	lb

Endogenous phase:

Part of total O_2 required	62.5	per cent
Quantity of O_2 needed	0.761	lb
Time required to oxidize 500 ppm cells produced in assimilation phase, (0.5-lb. cells)	160	hr
Hourly utilization of O_2	0.005	lb

Calculations for the tabulation above were based on an assimilation Q_{O_2}, of 100 and an endogenous Q_{O_2} of 6 obtained from more recent data (Hoover, *et al.*, 1952b, and unpublished work). A similar tabulation based on earlier data (Hoover and Porges, 1952) should be superseded by this one. An erroneous assumption in the earlier calculation alleged that a decrease in the amount of sludge produced would decrease the time required for complete oxidation. The fact is that endogenous respiration consumes a definite fraction of the amount present in unit time; for example, a Q_{O_2} of 10 is equal to a decrease of 1 per cent/hr., and one of 5 is equal to 0.5 per cent/hr. Thus the rate of autoxidation of sludge solids is proportional to the amount present and to the activity of the organisms. The time required is independent of the amount and inversely proportional to the rate.

Endogenous rates or Q_{O_2} of 5 to 16 were reported for many bacteria (Callow, 1924). A review of the literature by the authors showed similar rates for aquatic fauna. The rates reported were obtained with short experiments lasting only a few hours. The lower values are nearly typical of those in an operating plant with its daily cycle. In long-term experiments, the endogenous Q_{O_2} decreased to about 3 in 5 days and 1 to 2 in 20 days. The complete endogenous oxidation of sludge then must depend on operations that maintain a Q_{O_2} of about 6 for the daily cyclical process; a batch process would require a very long time.

If greater concentrations of microbial solids were carried in the aeration tank, the time required for assimilation would be less, but the rate of oxygen utilization would be greater. Thus 2500 ppm sludge solids would require a 5-fold increase of oxygen. In these experiments, the microbial solids content at the start was about 500 ppm. If high yields of cells were desirable for recovery of products such as vitamins (Hoover *et al.*, 1952a; Murdock, 1952), the crop would be harvested shortly after completion of the assimilation phase.

Complete combustion. Complete combustion of milk waste used 37.5 per cent of the oxygen requirement during assimilation, and this must be supplied within 6 hr under the assumed conditions. Oxygen must be provided at much greater rates than in the second or endogenous phase. Although the largest amount of oxygen (62.5 per cent) would be used in the latter step by the 500 ppm cell substance produced from the 1000 ppm skim milk solids, the oxygen would be required over a longer span of time.

The tabulation showed that conditions could be established to avoid accumulation of sludge or microbial cells. For example, if 2500 ppm microbial sludge were carried in the aerator under these conditions, 500 ppm would be destroyed by autodigestion. This amount of cell weight would be replaced by the cells formed on adding 1000 ppm skim milk solids. Under sufficient aeration and regulated feeding, this process would maintain a balance, producing no excess sludge and discharging an effluent practically free of organic matter. A plant based on such a process was empirically developed and proposed for waste disposal in small dairies (Thayer, 1951; 1952).

Research contracts. Research contracts have been negotiated with Pennsylvania State College for large-scale pilot plant studies on the rapid aerobic treatment of dairy wastes and also for a study of the effects on a stream receiving such treated wastes.

Summary

Disposal of dairy and other organic wastes by aeration is a branch of applied microbiology in which the biochemical oxidizing abilities of microorganisms are utilized. The microorganisms in the aerated mixture of wastes and sludge act rapidly on the available nutrients. Experiments made with a synthetic dairy waste, a balanced nutrient, showed that about 62.5 per cent of the COD (organic matter) is converted to cell material and the remainder is completely oxidized for energy. Continuing the aeration after the rapid synthesis (growth phase) favors autodigestion of the cell by endogenous respiration. Stoichiometric equations for the assimilative conversion of organic matter to cell substance and for the auto-oxidative digestion of cells explain these steps. During the period of assimilation, the oxygen demand of the organisms is high; oxygen is used at least 10 times the rate necessary in the second or endogenous phase, in which the organisms have a Q_{O_2} of 10 or less. The maintenance of aerobic conditions is mandatory during the oxidative processes and requires an oxygen tension of 0.3 to 0.5 ppm in solution. Under the conditions of these experiments, the assimilative phase is completed in 6 hr. In the endogenous phase, utilization of energy is much slower, requiring about 160 hr to oxidize the assimilated substance. The aeration process successfully converts high oxygen-demanding dairy wastes into cell substances of lower BOD.

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